studies involving the use of labeled precursors are planned to elucidate the mechanism involve

This is the initial report describing the cellular and biochemical changes which occur in th intestines of a euryhaline fish during seawater adaptation. In part, euryhaline fish overcome the desiccative effects of hypertonic seawater by drinking, and increasing the rate of monovalent ion transport across the intestines. We speculate that successful adaptation is dependent upon a compensatory growth response to this intensified physiological activity in the intestine.

Supported by USPHS Grant AM 06479 awarded to Dr. William Kinter, and NIH Grant 1 PO1 AM 09975 awarded to Dr. Bodil Schmidt-Nielsen.

1970 #21

DDT: ITS EFFECT ON INTESTINAL WATER ABSORPTION AND Na-K-ATPase IN TELEOSTS

Ralph Janicki, Jo Lingis, and William Kinter, Allegheny General Hospital, Pittsburgh, Pa., and the Upstate Medical Center, Syracuse, N. Y.

Teleosts are extremely sensitive to organochlorine pesticide poisoning (reviewed by Johnson, Trans. Am. Fish Co. 77:398-424, 1968). Unfortunately, pesticide concentrations in the marine environment continually increase due to agricultural run-off, a problem compounded by low pesticide biodegradability. In an attempt to elucidate the causal factors involved in organochlorine sensitivity in teleosts, the effect of DDT on osmoregulation is being investigated.

Marine and marine adapted euryhaline teleosts have overcome the problem of desiccation by their hypertonic environment. Tissue hypotonicity is maintained by drinking seawater and actively transporting salt, and with it water, across the intestinal epithelium Salts are eventua excreted by the gills; and water is retained.

The effect of DDT on intestinal salt and water absorption was measured in isolated gut sa of seawater adapted (2-3 weeks) eels (Anguilla rostrata), using a technique previously described by MacKay (Bull. MDIBL 9:23-26, 1969). DDT (10 mg/ml) was dissolved in N,N-dimeth formamide (DMF). The isotonic saline solution contained 50 parts per million (ppm) DDT, and 0.5% DMF. In these preparations, similar concentrations of dimethyl sulfoxide and ethanol had strong inhibitory effects on water absorption. All intestinal preparations were preincubated in their respective solutions for 1 hr, with repeated flushing, at 2-5° C. Eel guts were then incuba ed for 1 hr at 15° C; and water absorption was calculated as μl H_2O/g intestine x hr. Values ar the mean ± SE.

In 7 guts incubated in isotonic saline, H_2O absorption was 281 ± 30. In 7 guts incubated in isotonic saline containing 0.5% DMF, H_2O absorption was 250 ± 24, not significantly different from isotonic saline along ($P > 0.3$). However, 7 guts incubated in a fine suspension of 50 ppm DDT (1.4×10^{-4}M) in isotonic saline containing 0.5% DMF, H_2O absorption was reduced to 133 ± 26 (-47%; $P < 0.01$).

Water absorption is believed to passively follow active ionic transport. We therefore inves tigated the effect of DDT on intestinal mucosal Na-K-ATPase activity, the enzyme believed to be responsible for the transport of sodium across cell membranes. For a review see Skou (Physiol. Rev. 45:596-617, 1965).

Na-K-ATPase activity was measured on whole homogenates of intestinal mucosa using a

previously described procedure (Jampol and Epstein, Am. J. Physiol. 218:607-11, 1969); except that deoxycholate was omitted from the homogenization medium, and the reaction was run at 15° C. DDT darkened the Fiske and SubbaRow assay for inorganic phosphate (J. Biol. Chem. 66: 375-400, 1925). Therefore, after stopping the reaction with 30% TCA, DDT was extracted from a 3.0 ml aliquot of the supernatant with an equal volume of toluene. Some difficulty was encountered in securing a suitable solvent for DDT for the in vitro assay of Na-K-ATPase activity. For these studies, intestinal mucosal homogenates of the winter flounder (Pseudopleuronectes americanus) were used. At a final concentration of 5%, cyclohexanone, a common commercial solvent of DDT, completely inhibited Na-K-ATPase. Other solvents, at a final concentration of 5%, also had deleterious effects on in vitro Na-K-ATPase activity: acetone -33%; dimethyl sulfoxide -19%; and ethanol -34%. However, DMF had only a negligible 3% inhibitory effect on basal Na-K-ATPase activity.

Dose response curves were run on several species. At 50 ppm DDT, inhibition was: 63% in the eel (A. rostrata), see Figure 1; 38% in the winter flounder (P. americanus); 51% in the King O'Norway (Hemitripterus americanus); 37% in the yellow-tail flounder (Limanda ferruginea); and 35% in the long-horned sculpin (Myoxocephalus octodecimspinosus). Except in the eel (Figure 1), the Mg^{++} dependent ATPase was not affected by DDT.

Thus, low concentrations of DDT can impair the ionic dependent absorption of H_2O in the intestines of a marine-adapted euryhaline teleost. Since DDT inhibits Na-K-ATPase activity in

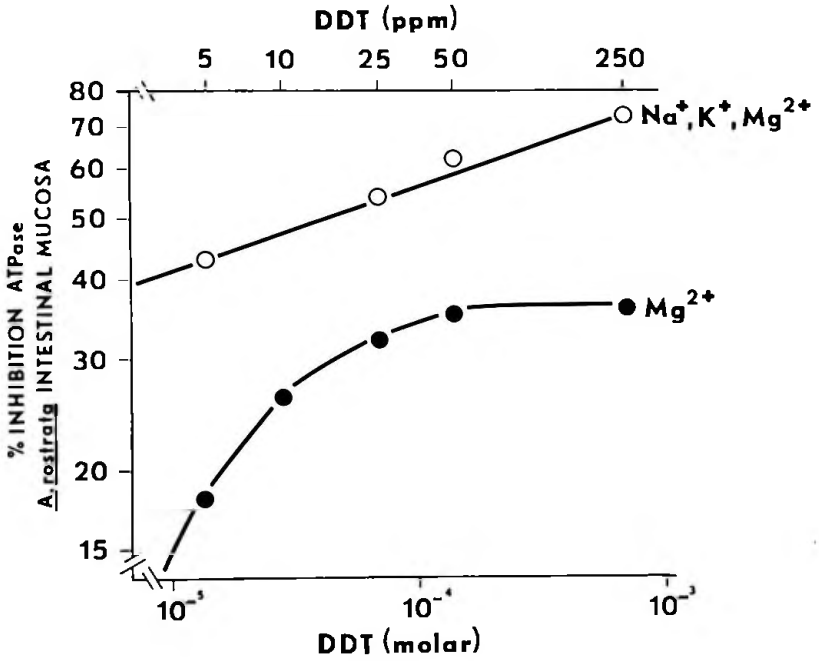

Figure 1. The effect of DDT on Na-K-ATPase amd Mg-ATPase in the intestinal mucosa of the eel (Anguilla rostrata). DDT concentrations range between 5 and 250 ppm. Efforts to determine whether DDT completely inhibits Na-K-ATPase have been unsuccessful, because suspensions above 250 ppm are too heavy. The break in the curve depicting the inhibition of Mg-ATPase suggests the presence of two Mg^{++} dependent enzymes.

intestinal mucosal homogenates, the inhibition of H_2O absorption can be explained at the biochemical level. The toxicity of DDT in teleosts may therefore be related to disrupted osmoregulatory transport mechanisms.

Of significance in this study is the inhibition of critical events in teleost osmoregulation by concentrations of DDT which can be expected to occur in these species in the foreseeable future. Filter feeding invertebrates are among the initial marine organisms which concentrate DDT. In the extracted fat of clams (Mya), collected in the relatively pesticide free waters of Frenchman Bay, 9 ppm DDT has been reported by Adamson, Sullivan and Rall (Bull. MDIBL 9:2-4, 1969). It is therefore disquieting that we report having found, on several occasions, clams of the genus Mya approximately 0.5 to 1.0 cm in diameter, in the intestines of the winter flounder (P. americanus). Thus, in one step of the food chain, an invertebrate filter feeder is providing a concentrated source of DDT to a commercially important fish.

Supported by grant AM 06479 from the USPHS.

1970 #22

TRANSPORT OF SUGARS INTO KIDNEY TUBULES OF THE FLOUNDER (Pseudopleuronectes americanus)

Arnost Kleinzeller and Leslie Hogben, Department of Physiology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

It has been reported previously (Bull. MDIBL 9:20, 1969) that 2-deoxy-D-glucose-^{14}C is accumulated in teased kidney tubules against a slight concentration gradient. The characteristics of this up-hill transport system have now been studied in more detail. Under standard conditions of incubation (1 h at 15 °C, air as a gaseous phase) of teased kidney tubules in an isotonic balanced saline (Forster, R. P., Science 108:65, 1948) buffered with a 5 mM TRIS-TES mixture, pH 7.2, the preparation had a mean water content of 5.5 kg H_2O/kg tissue dry wt (D.W.) (2 animals, 9 analyses). Mean value for tissue cations, in mequiv/kg D.W.: Na, 380; K, 308. Inulin-^{14}C space: 0.377 ± 0.011 (3 animals, 11 analyses). Figure 1 shows that, in contrast with D-galactose, the rate of accumulation of 2-deoxy-glucose (2-DGLU) does not reach a steac state by 90 min incubation. In this experiment, at an external sugar concentration of 1 mM, a tissue/medium (T/M) sugar ratio of 1.1 was reached after 90 min. The mean T/M in 6 experiments was 1.25 at 60 min. The following characteristics of the transport system for 2-DGLU were determined by studying the effects of various experimental conditions on the T/M after 60 min incubation:

1. The T/M was significantly depressed by 0.1 mM dinitrophenol, establishing the metabolic dependence of the transport process.

2. Accumulation of 2-DGLU was markedly enhanced by decreasing the medium concentration of the sugar.

3. D-glucose (medium concentration 5 mM) significantly depressed the T/M for 2-DGLU; D-galactose was less effective as a competitor whereas L-glucose was ineffective.

4. Phlorhizin and phloretin (both at 0.5 mM) were equally effective in inhibiting the transport of 2-DGLU.